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# *Aspergillus* species and mycotoxins: occurrence and importance in major food commodities

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*Aspergillus* species produce important mycotoxins, in particular aflatoxins, produced by *A. flavus* and related species, and ochratoxin A, produced by *A. ochraceus* and related species and also *A. carbonarius* and (less commonly) *A. niger*. In this review we briefly discuss the distribution of toxigenic *Aspergillus* species in nuts, coffee and cocoa beans, dried fruits, grapes, maize, rice and small grains. Future perspectives of distribution of *Aspergillus* species in foods are briefly discussed taking into account the impacts of climate change and the resilience of these mycotoxigenic species.

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## Introduction

*Aspergillus* is one of the three fungal genera most important in the spoilage of foodstuffs and in the production of mycotoxins, the others being *Fusarium* and *Penicillium*. *Aspergillus* species are the best adapted to growth in the tropics, as common species rarely grow below 10°C and most grow strongly at 37°C or above [1•]. Most species that occur commonly in foods are xerophilic, with major toxin producers all able to grow down to, or near to, 0.80 water activity [1•]. Some are strictly saprophytic, growing only after harvest, while some are commensals, able to grow in some plant crops and developing nuts or kernels before harvest without causing damage to the crop. *Aspergillus niger* is the only common species that is a serious pathogen, in some fruits and vegetables [1•]. However, *A. niger* strains only rarely produce mycotoxins [1•,2•].

Despite the ubiquitous occurrence of a wide range of *Aspergillus* species in tropical soils and vegetation, only a handful of species are significant mycotoxin producers in foods. The presence of mycotoxins in a food indicates that, at some stage in production or processing, conditions have been favourable for growth of a toxigenic fungus and mycotoxin production. The most important factor controlling the infection of foods by toxigenic *Aspergillus* species is whether infection can take place before harvest or only subsequently, during drying and storage. Species able to grow before harvest are commensals, that is, they have the ability to infect plants during growth without damaging the plant itself. That provides a ready mechanism for infecting nuts or grains before harvest. The importance of particular species varies with different foods, as will be briefly described in this review.

## *Aspergillus* species producing major mycotoxins in foods

The principal toxigenic *Aspergillus* species found in major commodities and the mycotoxins they produce are given in Table 1. Three species groups are represented: *A. flavus* and its close relatives; *A. ochraceus* and close relatives; and *A. carbonarius* and the closely related *A. niger*, although the latter is of much lower importance. All are classified in *Aspergillus* subgenus *Circumdati*. *A. flavus* and its close relatives, which produce aflatoxins, are commensal with peanuts and maize (and cotton, not discussed here). These species are able to grow in these plants under unfavourable growth conditions, such as drought stress, which permits infection of developing nuts or grains, and hence the production of aflatoxins before harvest. In the absence of such a plant–fungus association, infection and mycotoxin production occur only postharvest. *A. ochraceus* and closely related species, and *A. carbonarius* and *A. niger*, all of which produce ochratoxin A, have no known affinity with crop plants, so infection and mycotoxin production occurs only after harvest [3•]. Grapes are an apparent exception, as discussed below.

## Major commodities frequently contaminated with mycotoxins produced by *Aspergillus* species

Here we outline the distribution of toxigenic *Aspergillus* species in nuts, coffee, cocoa, dried fruits, grapes, rice, figs, maize and small grains.

### Nuts

The low sugar content of nuts means that small increases in moisture during storage or transport cause large

**Table 1**

**Distribution of toxigenic *Aspergillus* species in foods**

| Main source | Fungal species | Mycotoxins | References |
|---|---|---|---|
| Peanuts | *A. flavus* | Aflatoxins | [4–7] |
| | *A. parasiticus* | | |
| | *A. minisclerotigenes* | | |
| | *A. arachidicola* | | |
| | *A. niger* | Ochratoxin A | [8,9] |
| Tree nuts (almonds, pistachios, walnuts, hazelnuts) | *A. flavus* | Aflatoxins | [10,11] |
| | *A. carbonarius* | Ochratoxin A | [12] |
| Brazil nuts | *A. flavus* | Aflatoxins | [11,13] |
| | *A. parasiticus* | | |
| | *A. nomius* | | |
| | *A. arachidicola* | | |
| | *A. bombycis* | | |
| | *A. pseudonomius* | | |
| | *A. pseudotamarii* | | |
| Coffee | *A. westerdijkiae* | Ochratoxin A | [15–18] |
| | *A. ochraceus* | | |
| | *A. steynii* | | |
| | *A. carbonarius* | | |
| | *A. niger* | | |
| Cocoa | *A. flavus* | Aflatoxins | [23] |
| | *A. parasiticus* | | |
| | *A. nomius* | | |
| | *A. westerdijkiae* | Ochratoxin A | [20–22] |
| | *A. ochraceus* | | |
| | *A. melleus* | | |
| | *A. carbonarius* | | |
| | *A. niger* | | |
| Dried fruits | *A. flavus* | Aflatoxins | [25,26] |
| | *A. ochraceus* | Ochratoxin A | [2•] |
| | *A. carbonarius* | | |
| | *A. niger* | | |
| Dried figs | *A. flavus* | Aflatoxins | [25,26] |
| | *A. ochraceus* | Ochratoxin A | [2•] |
| | *A. carbonarius* | | |
| | *A. niger* | | |
| Grapes | *A. carbonarius* | Ochratoxin A | [27–29] |
| | *A. niger* | | |
| Maize | *A. flavus* | Aflatoxins | [30–32] |
| | *A. parasiticus* | | |
| | *A. arachidicola* | | |
| | *A novoparasiticus* | | |
| | *A. pseudocaelatus* | | |
| | *A. ochraceus* | Ochratoxin A | [34] |
| | *A. carbonarius* | | |
| | *A. niger* | | |
| Rice | *A. flavus* | Aflatoxins | [35•,36] |
| | *A. caelatus* | | |
| | *A. novoparasiticus* | | |
| | *A. arachidicola* | | |
| | *A. pseudocaelatus* | | |
| Small grains | *A. flavus* | Aflatoxins | [1•,3•] |
| | *A. ochraceus* | Ochratoxin A | [1•,37] |

increases in water activity, increasing the possibility of mycotoxin formation. In addition, their high oil content appears to favour aflatoxin production, so affected nuts frequently contain high levels of this toxin.

## Peanuts

Because *A. flavus* and *A. parasiticus* are commensals in the peanut plant [3•,4] these nuts have a much higher risk of serious contamination with aflatoxin than tree nuts. Indeed *A. parasiticus* appears to have a specific association with peanuts [4] and has been isolated in Egypt [5] and Brazil [6], yet is of uncommon occurrence in other foods. Moreover, it is rarely isolated, if at all, in Southeast Asia [4]. *A. minisclerotigenes* has only been isolated from peanuts [7], but whether that is due to a specific association is unknown.

Peanuts are frequently infected by these species while still in the ground, and if the crop suffers drought stress or related factors, unacceptable levels of aflatoxins may be produced before harvest [3•]. Slow drying and poor storage conditions are a serious issue in the humid tropics, as moisture absorption may cause large increases in aflatoxin levels after harvest [3•,[3•]].

Although species classified in *Aspergillus* section *Nigri* are common in peanuts [4,8], ochratoxin A concentrations are insignificant in comparison with aflatoxins [8,9]. Observations have shown that, unlike *A. flavus*, *A. niger* infections in peanut kernels cause complete destruction of the kernel, resulting in empty shells only at harvest (JI Pitt, unpublished observations).

## Tree nuts

Tree nuts (almonds, pistachios, walnuts and brazil nuts) sometimes have high levels of infection by *A. flavus* and hence unacceptable levels of aflatoxins [3•,10,11]. In general, the range of toxigenic *A. flavus* and related species found in tree nuts is similar to that of peanuts. As *A. flavus* has no affinity with nut trees, the fungus infects postharvest unless insect damage causes preharvest infection or, in pistachios, early splitting of the hulls allows entry of the fungus while nuts are still moist. Good agricultural and manufacturing practice limit aflatoxin formation in tree nuts, except for Brazil nuts.

Levels of ochratoxin A in tree nuts are usually very low, though occasional samples have unacceptable concentrations [12].

## Brazil nuts

A major challenge for brazil nut production is the control of contamination by aflatoxigenic fungi and aflatoxins [11,13]. The Amazon rainforest favours a unique biodiversity of fungal species different from cultivated crops [14•]. Brazil nuts have been found to contain a wider range of toxigenic species than other tree nuts, and the infection of Brazil nuts by species from *Aspergillus* section *Flavi* can reach 100% [11]. Brazil nut trees are very tall, so harvest relies on the nuts falling to the ground, where they lie until conditions for gathering are favorable, often permitting time for *A. flavus* infection. As the trees occur in natural forests, good agricultural practice does not apply there. Species of *Aspergillus* isolated from Brazil nuts and capable of producing aflatoxins were *A. flavus*, *A. nomius*, *A. pseudonomius*, *A. bombycis*, *A. arachidicola* and *A. pseudotamarii* [11,13]. *A. nomius* has been recognized as a major source of aflatoxins in Brazil nuts, as 100% of isolates were able to produce aflatoxins B and G [11,13].

## Coffee

The possibility that coffee could contain ochratoxin A was first reported in the 1970s [15]. However, recognition that *A. ochraceus* (and related species) and *A. carbonarius* were the sources of ochratoxin A did not occur until 30 years later [16]. *A. ochraceus* was subsequently split into three species and one of these, *A. westerdijkiae*, is now recognized as the main source of ochratoxin A in arabica coffee [17], while *A. carbonarius* is more important in robusta coffee [18]. Infection of coffee beans by toxigenic *Aspergillus* species does not occur until the drying stage [16].

Reports of *A. flavus* or related species in coffee beans have been uncommon, and aflatoxin is not considered to be a problem in coffee.

## Cocoa

Although ochratoxin A was first reported from cocoa beans in 1973 [19] and effective analytical methodology developed by 1983 [20], the first major survey for the presence of this toxin on cocoa and cocoa products only occurred in 2004 [21]. Levels were uniformly low.

During fermentation, the occurrence of *A. flavus*, *A. parasiticus*, *A. carbonarius* and *A. niger* have been reported [22,23]. Sun drying is the common drying process, taking seven days under good conditions but up to four weeks if the weather is adverse, increasing the likelihood of fungal growth and aflatoxin and ochratoxin A formation.

Copetti *et al.* [24] demonstrated that a good fermentation stage, when lactic acid bacteria produce organic acids, especially acetic acid, minimises the growth of ochratoxigenic fungi. They also showed that the fermentation of partially depulped beans during drying could increase ochratoxin A production.

Although *A. flavus* and related species have been isolated from cocoa fermentations, aflatoxin levels always remain low as conditions for *A. flavus* growth appear to be unfavourable [23].

### Dried fruits

As tree fruits are usually preserved by sun drying, the most commonly isolated fungi are the black Aspergilli, *A. niger* and *A. carbonarius*, which possess a high resistance to UV light and sunlight, due to their pigmentation [1•,2•]. Species related to *A. flavus* and *A. ochraceus* are therefore uncommon in dried tree fruits. In addition, peaches, apricots and pears are usually treated with high levels of sulphur dioxide, which not only prevents nonenzymic browning and preserves colour, but also renders the fruit sterile.

### Figs

Figs are sometimes infected by *A. flavus* [25]. The unique structure of the fruit evolved to enable fertilization by insects which carry *A. flavus* spores into the seed cavity. Also, fallen figs are harvested from the ground which can add to the contamination problems in some countries. Immature figs are not colonized by *A. flavus*, but once they are ripe infection occurs readily and fungal growth continues during drying [26]. Sorting of individual figs by UV light has been used to identify and remove those heavily contaminated, decreasing the overall aflatoxin load [3•].

### Grapes

Maturing grapes have high acidity and sugar contents, which make them ideal substrates for many *Aspergillus* species. High temperatures and sunlight during grape maturation ensure that the black Aspergilli are common inhabitants of vineyards. However, it does not appear that Aspergilli can penetrate intact grape skins, as they are not pathogens. Entry to maturing grapes results from attack by pathogenic fungi such as *Rhizopus stolonifer*, *Botrytis cinerea* or powdery mildews, from mechanical damage due to cultivating or harvesting equipment, or, in some cultivars, from the splitting of berry skins from rain events near harvest time. Once entry to a berry is gained, the black Aspergilli thrive in the acidic, high sugar environment [3•]. Growth of *A. carbonarius* and *A. niger* is common in harvested grapes, resulting in ochratoxin A formation during sun drying. The black Aspergilli will continue to grow and produce ochratoxin A until the grapes dry to $<0.80$ water activity [27]. *A. carbonarius* is the significant species for ochratoxin A formation in grapes and grape products: this toxin is produced by only a low percentage of *A. niger* isolates and not at all by those of *A. japonicas* and others uniseriate black aspergilli [2•,28].

In grapes used for wine making, the anaerobic conditions achieved during fermentation stops fungal growth and toxin production. So control of ochratoxin A formation in wines relies on good vineyard management, that is, control of bunch rots and skin splitting, and a short time interval between harvest and crushing [29].

For the reasons outlined above, infection of grapes by *A. flavus* and related species is uncommon, so aflatoxin does not normally occur in grapes or grape products.

### Maize

Aflatoxin formation remains a major issue with maize crops worldwide [30–32]. Like peanuts, maize appears to have a commensal relationship with *A. flavus* although, perhaps surprisingly, not with *A. parasiticus*. Consequently, *A. flavus* infections and aflatoxin B production in maize is common, but *A. parasiticus* infections are rare [4]. As is the case with peanuts, the soil in which maize is grown is often highly contaminated with *A. flavus* sclerotia (resting bodies) and conidia, as the result of colonisation of unharvested grains or kernels. These particles provide a ready source of inoculation of future crops [33], entering developing cobs either during silking or by insect damage, providing access to ripening kernels. Colonisation of the silks also allows invasion of the cobs directly [33]. Maize is particularly sensitive to drought stress, which increases *A. flavus* density in soil and reduces the plant defense mechanisms. Like peanuts, aflatoxin formation can occur during the late stages of growth, during poor drying and storage. Using molecular techniques, Viaro *et al.* [32] recently detected the occurrence of five aflatoxigenic *Aspergillus* section *Flavi* species in maize. For the first time, *A. novoparasiticus*, *A. arachidicola* and *A. pseudocaelatus*, all aflatoxin B and G producing species, were found, in addition to *A. flavus* and *A. parasiticus*. However, *A. flavus* was overwhelmingly the dominant species found.

*A. niger* and presumably *A. carbonarius* also commonly occur in maize. Ochratoxin A has occasionally been reported from maize and maize products, but at very low levels [34]. *A. ochraceus* and related species are not associated with freshly harvested maize, but are sometimes able to grow and produce ochratoxin A during long storage [1•].

### Rice

In a recent study carried out on Brazilian rice, five species were distinguished from *Aspergillus* section *Flavi*: *A. flavus*, *A. caelatus*, *A. novoparasiticus*, *A. arachidicola* and *A. pseudocaelatus* [35•]. This was the first report of these last three species from rice and rice plantation soil. Only a low percentage (1.5%) of isolates of *A. flavus* from rice were able to produce aflatoxins in culture, in contrast to other major crops where a higher proportion of *A. flavus* producing aflatoxins have been reported, including peanuts 50% [[35•]], brazil nuts 46% [11], and maize 70% [33]. Although most of the *A. flavus* isolated in this study did not produce aflatoxins, 69% produced cyclopiazonic acid [35•].

Because *A. flavus* has no affinity with rice plants, aflatoxin formation in rice occurs only postharvest, and it is usually found at only low levels [4]. Recent studies of paddy and brown rice inoculated with *A. flavus* spores showed that while significant dry matter loss occurred in brown rice with concomitant aflatoxin $B_1$ production, significantly

less dry matter loss and aflatoxin $B_1$ was produced in paddy rice. This supports the possibility that the rice kernel is protected in some way before threshing, which would minimize aflatoxin contamination [36].

### Small grains

Small grains (wheat, barley, oats and triticale) have no affinity with the toxigenic *Aspergillus* species, so aflatoxin and ochratoxin A are not present at harvest. These toxins can accumulate subsequently due to slow drying or poor storage [3•]. In cool temperate climates, ochratoxin A in small grains is almost always the result of the growth of *Penicillium verrucosum* postharvest [1•]. Indeed, recent studies in Canada suggest that *P. verrucosum* colonizes grain at inlets and outlets in silos especially during the spring months which allows ochratoxin A contamination to occur [37].

## Future perspectives and conclusion

In this brief review the distribution of toxigenic *Aspergillus* species in some foods is discussed. In the future the effect of climate change may affect growth of these toxigenic fungi and hence mycotoxin production, as environmental conditions including temperature, relative humidity, increased $CO_2$ and sunlight affect their survival. The climate change effects on fungal survival and mycotoxin production have been discussed elsewhere [38•]. Certainly, interactions between these three factors have been shown to stimulate aflatoxin B production by *A. flavus*, and RNAseq has shown that gene clusters related to secondary metabolite production, stress related functional genes and sugar transporters amongst others were important [39].

In our opinion, if the temperature increases and water supply decreases in the future, the adaptation of fungi by mutation and sexual recombination would give some advantages for survival of resilient species. Warmer climates favour thermotolerant species, and this will lead to dominance by toxigenic *Aspergillus* species over *Penicillium* species as well as perhaps changing the relative production of mycotoxins by the same species, for example, *A. flavus* and aflatoxins and cyclopiazonic acid. Drought stress is beneficial to xerophilic species such as *A. flavus* which thrive in lower rainfall and higher temperature conditions. Thus fungal community structure and diversity may change significantly influencing the relative mycotoxin contamination of these commodities.

## Conflict of interest

There are no conflicts of interest.

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- • of special interest

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In this recent work,*Aspergillus* section *Flavi* were isolated from rice throughout supply chain and soil samples. Using a polyphasic approach, with phenotypic (morphology and extrolite profiles) and molecular data (beta-tubulin gene sequences), five species were identified: *A. flavus*, *A. caelatus*, *A. novoparasiticus*, *A. arachidicola* and *A. pseudocaelatus*.

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This interesting review examines the available evidence on the impacts of interacting climate changes factors on growth and mycotoxin production by key toxigenic fungi including*Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* species.

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